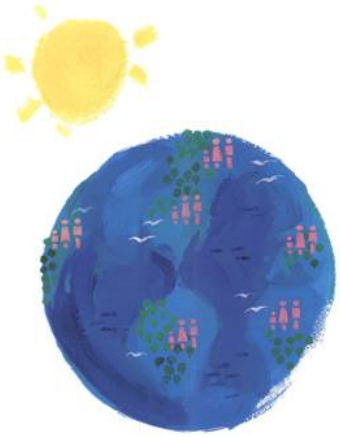
NICEATM

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

ICCVAM

Interagency Coordinating Committee on the Validation of Alternative Methods



Nominations to ICCVAM

Richard McFarland, M.D., Ph.D., FDA, ICCVAM-PWG Chair

Jodie Kulpa-Eddy, D.V.M., USDA, ICCVAM Chair

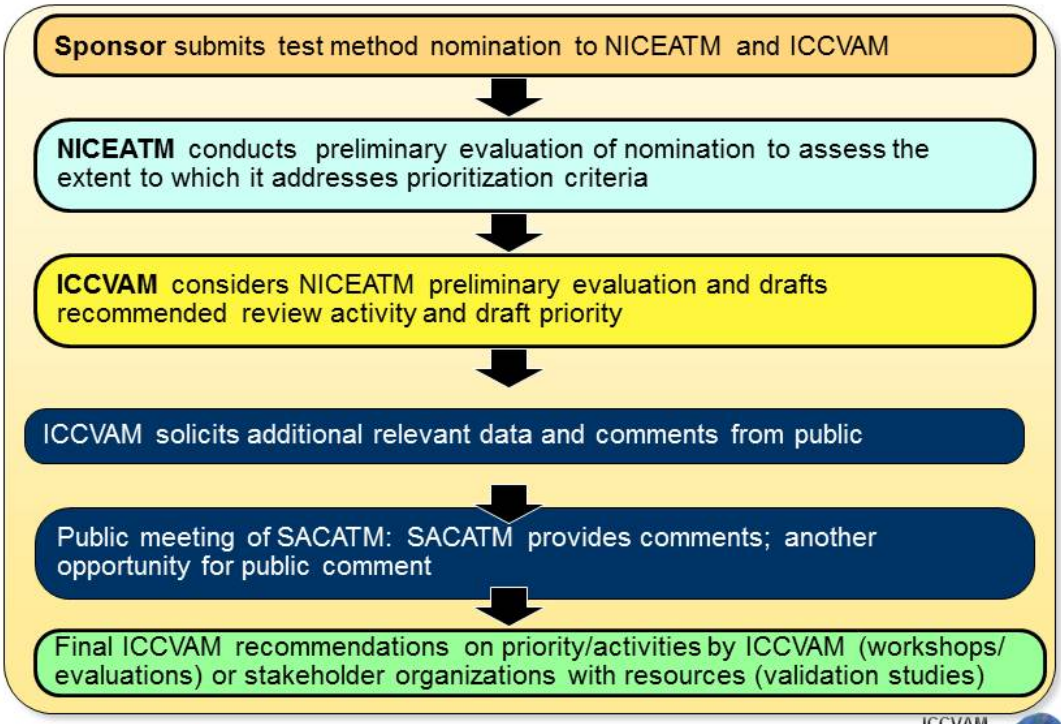
SACATM Meeting
June 16, 2011
Hilton Arlington,
Arlington, VA

Nominations to NICEATM and ICCVAM

- Nominations include
  - Test methods proposed for validation studies that appear promising based on available data
  - Test methods recommended for a workshop or other activity
  - Test methods proposed for ICCVAM evaluation that have completed validation studies, but lack a complete submission package and background review documents

More information and instructions on submitting a nomination or submission to NICEATM and ICCVAM are available at http://iccvam.niehs.nih.gov/SuppDocs/submission.htm

Process for Nominations of Test Methods to ICCVAM



ICCVAM Criteria for Prioritization

1. Applicability to regulatory testing needs and agency programs
2. Potential to reduce, refine, and replace animal use compared to currently accepted method(s)
3. Extent of expected use or application and impact on human, animal, or ecological health
4. Potential for improved prediction of adverse health or environmental effects, compared to currently accepted method(s).
5. Other advantages compared to currently accepted method(s) (e.g., reduced cost and time to perform).

NICEATM — *National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods*

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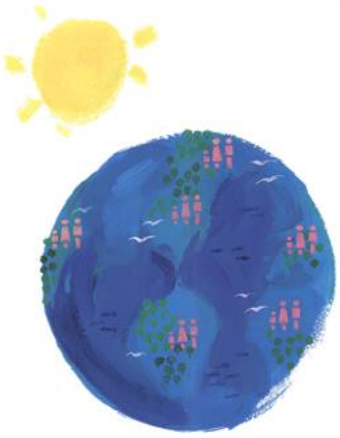


Nomination of an *In Vitro* Pyrogen Test Method for Assessing Non-Endotoxin Pyrogens

Richard McFarland, M.D., Ph.D.,
CBER, FDA
ICCVAM Pyrogenicity Working Group Chair

SACATM Meeting

June 16, 2011

Hilton Arlington
Arlington, VA

Pyrogen Testing

- Pyrogens = substances that induce fever
- May originate from a variety of biological or synthetic/manufacturing sources
- May also be released from microbiological organisms such as bacteria, viruses, and fungi during cell death or following immunological attack
- Bacterial endotoxin: one of the most potent pyrogenic materials; outer membrane component of the Gram-negative bacteria cell wall
- Pyrogens may also be found in processing and packaging materials, chemicals, raw materials, or equipment used during the manufacturing of parenteral drugs or medical devices.
  - Also includes non-endotoxin pyrogens
- Pyrogen testing is primarily used by regulatory authorities for end-product release of human and animal parenteral drugs, biological products, and medical devices
  - Results are used to limit the risks of febrile reaction (which can result in death) in the patient

In Vitro Pyrogen Test Methods Reviewed in the Previous ICCVAM Evaluation

- All are based on primary human blood/cells or a human cell line:
  - The Human Whole Blood (WB)/Interleukin (IL)-1β *In Vitro* Pyrogen Test
  - *The Human WB/IL-1β In Vitro Pyrogen Test: Application of Cryopreserved (Cryo) Human WB*
  - The Human WB/IL-6 *In Vitro* Pyrogen Test
  - The Human Peripheral Blood Mononuclear Cell (PBMC)/IL-6 *In Vitro* Pyrogen Test
  - The Monocytoid Cell Line Mono Mac 6 (MM6)/IL-6 *In Vitro* Pyrogen Test

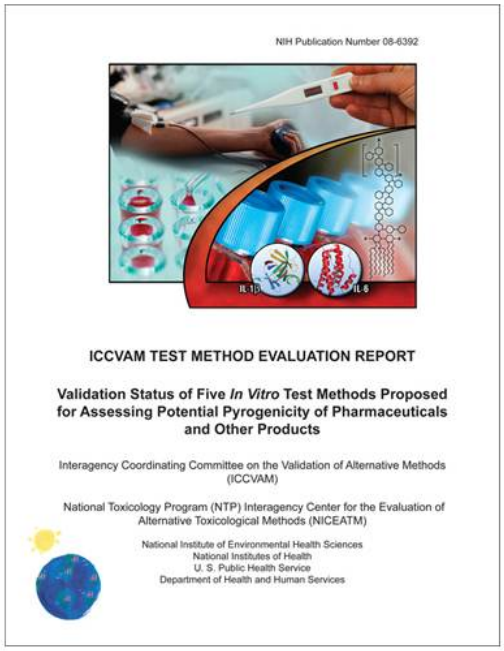
NICEATM-ICCVAM Peer Review Panel Meeting

- February 6, 2007
  - NIH, Bethesda, MD
- Expert Scientific Panel
  - 13 scientists
  - 5 countries
- Range of expertise includes:
  - Immunology
  - Microbiology
  - *In vivo* and *in vitro* pyrogen testing
  - Biostatistics
  - Test method validation

In Vitro Pyrogenicity Peer Review Panel

- **Karen Brown, Ph.D. (Panel Chair)**
  DRL Pharma & Pair O' Doc's Enterprises
  Parkville, Missouri
- **Brian Crowe, Ph.D.**
  Baxter Vaccine AG
  Orth an der Donau, Austria
- **Nancy Fluornoy, Ph.D.**
  University of Missouri-Columbia
  Columbia, Missouri
- **Ihsan Gursel, Ph.D.**
  Bilkent University
  Bilkent, Ankara, Turkey
- **Ken Ishii, MD, Ph.D.**
  Osaka University
  Osaka, Japan
- **Jack Levin, M.D.**
  University of California San Francisco
  San Francisco, California
- **Albert Li, Ph.D.**
  In Vitro ADMET Laboratories
  Rockville, Maryland
- **David Lovell, Ph.D.**
  University of Surrey
  Guilford, United Kingdom
- **Melvyn Lynn, Ph.D.**
  Eisai Medical Research, Inc.
  Teaneck, New Jersey
- **Anthony Mire-Sluis, Ph.D.**
  AMGEN, Inc.
  Thousand Oaks, California
- **Jon Richmond, M.D.**
  Animals Scientific Procedures Division
  Tayside, United Kingdom
- **Peter Theran, V.M.D.**
  Massachusetts Society for the Prevention of Cruelty to Animals
  Novato, California
- **Kevin Williams**
  Eli Lilly
  Indianapolis, Indianapolis

2008 ICCVAM Recommendations: In vitro pyrogen tests


NIH Publication Number 08-6392

ICCVAM TEST METHOD EVALUATION REPORT

Validation Status of Five *In Vitro* Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences
National Institutes of Health
U. S. Public Health Service
Department of Health and Human Services

- Usefulness and Limitations
  - Can detect Gram-negative endotoxin
    - Case-by-case basis, subject to product-specific validation
  - **Scientific basis suggests they can detect non-endotoxin pyrogens; insufficient data to support this broader application**
- Future Studies
  - Include endotoxin-spiked and non-endotoxin spiked samples
  - Include 3-way parallel testing (*in vitro*, RPT, and BET)
  - Include a broader range of test substances (i.e., more than just parenteral pharmaceuticals)

2009 Federal Agency Responses

- ICCVAM recommendations endorsed by Federal agencies that regulate pyrogenicity testing
- *These in vitro pyrogen test methods may be considered on a case by case basis for the detection of Gram negative endotoxin in parenteral drugs, subject to product-specific validation (FDA, April 22, 2009)*

2011 Nomination from Biotest AG: Monocyte Activation Test (MAT)-Cryopreserved

- MAT is one of the methods previously reviewed by ICCVAM
  - Based on quantifying interleukin (IL)-1β release from cryopreserved human blood cells
- Nominated for coordination of an independent validation study to evaluate the MAT for its ability to detect non-endotoxin pyrogens
  - Proposed validation study would involve the analysis of samples spiked with endotoxin and non-endotoxin pyrogens
  - Lipoteichoic acid (LTA) is proposed as the non-endotoxin standard; crude preparations from Gram-positive bacteria will also be considered
  - Directly addresses ICCVAM's previous recommendations

Prioritization Criterion 1: Potential Applicability to Regulatory Testing Needs and Agency Programs

Agency, Center, or Office	Regulated Products	Statutory Requirements	Relevant Guidelines and Guidances
FDA-CBER	Biological products	Federal Food, Drug, and Cosmetic Act (U.S.C. Title 21, Chapter 9)	USP34 NF29<85> USP34 NF29<151> ISO 10993-11
FDA-CDER	Human parenteral pharmaceuticals		
FDA-CDRH	Medical devices		
FDA-CVM	Veterinary pharmaceuticals		

[1]Mechanisms exist for test method developers to qualify their method on a case-by-case basis. The use of any recommended method will be subject to product-specific validation to demonstrate equivalence as recommended by the FDA (e.g., 21 CFR 610.9 and 21 CFR 314.50(d)(1)(ii)(a)).

Prioritization Criterion 2. Potential to Reduce, Refine, Replace Animal Use

- Rabbit pyrogen test (RPT) reportedly uses >300,000 rabbits per year
- Up to 15% mortality among horseshoe crabs used for bacterial endotoxin test (BET)
- MAT uses human blood samples (cryopreserved)
  - Already accepted as a replacement for the RPT for Gram-negative endotoxin testing
  - Availability for non-endotoxin pyrogens will further reduce and replace use of animals

Prioritization Criterion 3. Extent of Expected Use or Application and Impact on Human, Animal, Or Ecological Health

- The response detected in the MAT model system is expected to more closely predict the human response than nonhuman test systems due to use of human cells involved in the fever reaction

Prioritization Criterion 4. Potential to Provide Improved Prediction of Adverse Health or Environmental Effects

- MAT uses human blood cells and therefore may reflect the human physiological response better than the RPT and BET
- Directly analyzes effects of pyrogens on cells that regulate the inflammatory responses in the human body
- A number of substances are incompatible with the RPT or BET, but the *MAT can be used for these:*
  - Drugs that influence temperature regulation
  - Drugs that cause immunological reactions
  - Cellular preparations and medical devices
  - Drugs interfering with the clotting system or substances masking lipopolysaccharide cause BET testing problems

Prioritization Criterion 5. Other Advantages Provided by MAT

- Uses cryopreserved human blood
  - Allows more constant availability in the laboratory in standardized form
  - Can be pretested for possible blood-borne pathogens
  - *Results in improved logistics and a reduction of the time from experimental concepts to results*

ICCVAM Proposal for Prioritization and Activities

- Nominated activity should be a high priority
- Further discussion should proceed to determine what additional information is needed to adequately characterize the usefulness and limitations of the MAT for identifying non-endotoxin pyrogens
  - Will require an assessment of what data are needed and what studies are required to fill any data gaps
  - Studies identified that are considered necessary to adequately characterize its validation status for regulatory testing purposes should also be considered high priority

Questions for SACATM

1. With regard to the nomination of Biotest's Monocyte-Activation Test for detection of non-endotoxin pyrogen contamination in pharmaceutical products, medical devices, and other samples as an alternative to further replace the rabbit pyrogen test, please comment on the proposed ICCVAM priority and activities. Do you agree with the proposed priority and activities, or if not, please explain?